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Adult Age and Breeding Structure of a Hawaiian *Drosophila silvestris* (Diptera: Drosophilidae) Population Assessed via Female Reproductive Status[1]

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ABSTRACT: The Upper 'Ōla'a Forest population of *Drosophila silvestris,* a dipteran species endemic to the island of Hawai'i, was studied to investigate adult age and breeding structure of this natural population. Analyses of insemination status and ovarian developmental stage were carried out for both laboratory-reared and field-collected females, including a sample of F_1 individuals that had been marked and released into the field population shortly after adult eclosion. Marked females were recaptured from 7 days old to more than 4 months after release; this sample included representatives of all seven ovarian developmental stages scored (from early previtellogenesis to fully mature ovaries). The profile of female reproductive maturation in the field flies was similar to that in laboratory-reared flies, except that developmental rates were substantially slower and more variable in the natural population, largely because of lower field temperatures. Using information on ages and ovarian condition of the marked females, an independent population sample of wild-caught adult females was estimated to include 28% young flies approximately 2 to 3 weeks old (ovaries previtellogenic), 37% maturing flies from 2 to 4 or more weeks old (vitellogenic ovaries), and 35% reproductively mature flies from 1 to more than 4 months old. The unexpected excess of young flies in the adult population up to 4 or 5 weeks old (65%) can be interpreted by several alternative hypotheses (e.g., age-related dispersal, predation, location of suitable breeding substrates, baiting effects), but further studies are required to confirm whether this age pattern is typical. Earliest onset of female receptivity occurred at mid vitellogenesis in both field and laboratory flies, with insemination frequencies increasing as ovaries matured. It is surprising that field females showed higher mating success at all competent ovarian stages than females reared in the continuous presence of males. Further, all reproductively mature field females, both marked and unmarked, were inseminated. In this species, sexual selection acts primarily on males, with the lack of female mating failure in the field providing no evidence of sexual selection among adult females.

REPRODUCTIVE PATTERNS in natural populations influence the size, genetic structure, and evolutionary potential of such populations and are therefore central to any analysis of microevolutionary processes in nature. The endemic Hawaiian drosophilids, with their spectacular adaptive radiation and rapid speciation (Carson et al. 1970, Kambysellis and Heed 1971, Kambysellis and Craddock 1997), provide a unique opportunity to investigate the forces underlying evolutionary divergence and so begin to understand insular evolution. To do so and to evaluate various population genetic models realistically requires knowledge of the ecology, life history traits, mating structure, reproductive

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potential, and approximate population sizes of an array of related species, but rarely is all this information available, even for one species. To this end, we have combined an ecological approach with a developmental analysis of reproductive maturity to learn more about the structure of a local population of one particular Hawaiian *Drosophila* species.

Drosophila silvestris is a long-lived "picture-winged" fly endemic to Hawai'i, the youngest island in the Hawaiian Archipelago. This species has been the focus of population analyses for over 20 yr and indeed is the best studied of the more than 800 endemic species. Because of past and ongoing volcanic activity on Hawai'i, *D. silvestris* populations have a defined spatial structure with marked geographic variation in inversion and allozyme frequencies between disjunct isolates (Craddock and Johnson 1979, Craddock and Carson 1989), and a distinct temporal structure as well, resulting from extinction and recolonization of local populations coincident with episodes of volcanic activity (Carson et al. 1990). Dissection of ecologically suitable tracts of montane rain forest by lava flows and ash deposits restricts this species' distribution to a few *kīpuka*s and larger patches of forest, composing less than 700 km^2 overall (Craddock and Carson 1989). These geographic and temporal factors, combined with frequent founder events in the history of the species, contribute to a dynamic population stucture. Because *D. silvestris* is locally rare and somewhat difficult to collect, the impression has been that sizes of local demes are relatively small. However, no direct estimates of population densities have previously been undertaken.

Drosophila silvestris is a relatively large fly with an average thorax length of 3.2 mm. Ovaries of mature field females have, on average, 52 ovarioles per fly and one to three mature eggs per ovariole, giving this species a high fecundity potential (Kambysellis and Heed 1971). If suitable oviposition and breeding substrates are abundant, and adult nutrition is not limiting, thousands of progeny should result from each fertilized female. Individuals live for many months and oogenesis, once begun, is continuous with synchronous ovariole development. Hence, this species has a high potential for increase, characteristic of an *r*-selected species (MacArthur and Wilson 1967). This considerable reproductive potential validates the role of founder-flush cycles (Carson 1971) in the geographic spread and evolutionary divergence of the Hawaiian *Drosophila.* What presents a paradox is the perceived small sizes of current populations in the face of the predicted high reproductive potential of this and other Hawaiian "picture-winged" species with similar ovarian function.

Populations may conceivably be limited by the availability of resources appropriate to the various life history stages, as well as by peculiarities of the reproductive system. For instance, sexual maturity may be greatly delayed, restricting the period of active reproduction, or perhaps only a few of the surviving adults actually mate and produce progeny. To determine whether there are any reproductive anomalies in *D. silvestris,* we examined adult individuals from Upper 'Ōla'a Forest, in conjunction with a mark-release-recapture study of this population (W.D., unpubl. data).

Here, we report on the ovarian developmental condition and rates of reproductive maturation of females taken from this population, and use these developmental data in conjunction with laboratory data to assess adult age distribution in the field population. In *Drosophila* oogenesis (King 1970), the most dramatic growth in the ovaries takes place during vitellogenesis, the stage of synthesis and uptake of vitellogenins or yolk proteins. Ovaries as a whole as well as individual egg chambers or follicles can be readily classified as previtellogenic, vitellogenic, or postvitellogenic, providing some measure of the individual's developmental age. Examination of the female reproductive system for the presence of stored sperm can also reveal whether the individual has mated and can contribute to the next generation. Such data from a field population, when compared with laboratory data on reproductive maturation and mating, provide further insights into the breeding structure and reproductive pattern of *D. silvestris,* relevant

to its past differentiation and evolutionary potential.

MATERIALS AND METHODS

The Study Population

The natural population of *D. silvestris* selected for study is within Upper 'Ōla'a Forest Reserve (locality code Y-11) at the end of Wright Road, in a region of continuous tree-fern forest containing one of this species' main host plants, *Clermontia. Drosophila silvestris* females were also observed ovipositing on a decaying log of *Cheirodendron.* The fieldwork of Dominey in this area was carried out over the course of about 1 yr, beginning in August 1986. Collecting stations were distributed along an altitudinal transect with fermenting mushroom and/or banana baits set up every 50-m inclination up a pig hunters' trail, beginning at ~1150 m. The prime site was at 1350 m altitude. The winter of 1986–1987 was unusually cool, with a recorded minimum in 'Ōla'a of only 3°C; daytime temperatures were generally above 10°C. Nonetheless, there was a vigorous population of *D. silvestris* present throughout the year.

Rearing Procedures

Approximately 100 wild-caught females, inseminated in nature at the Y-11 site, were maintained at Hawai'i Volcanoes National Park for several months and allowed to oviposit on Wheeler-Clayton medium (Wheeler and Clayton 1965) in vials held in 3.785-liter (gallon) jars. Food vials were replaced weekly. Because facilities at Volcano were inadequate for the special care and handling entailed in rearing Hawaiian *Drosophila,* vials containing newly oviposited eggs were shipped weekly to the University of Hawai'i (UH) Hawaiian *Drosophila* laboratory for rearing of the F_1 progeny via standard procedures (Carson 1987). Newly eclosing adults were collected from the sand jars twice weekly and shipped back to Volcano for marking and field release at the study site.

Marking Procedures

Laboratory-reared adults, the F_1 progeny of wild-caught inseminated females taken at the Y-11 site, were marked within a week of eclosion, on receipt of each shipment from Honolulu. The flies were cooled at 4°C for a few minutes to immobilize them and then coded by marking them with one to four spots of either white, red, blue, green, or yellow enamel paint before release at the study site.

As a control, some of the marked F_1 adult flies were not released; instead they were maintained in a shaded location at Volcano in a garbage can that had netting on the side to admit light. Daily temperature maxima and minima were recorded in the immediate vicinity of the can.

Ovarian Developmental Stage and Insemination Status

Adult females (marked and unmarked) collected from the study site were placed singly into small capsules and frozen within 2 hr of capture. Marked F_1 females sampled from those held in captivity at Volcano were likewise frozen, and the numbered frozen specimens were shipped on ice to UH where they were immediately placed into the freezer. Females were dissected in saline and their ovaries and sperm storage organs examined as previously described (Kambysellis and Craddock 1991). For each individual, the ovarian stage was determined by noting the developmental condition of the most advanced oocytes (i.e., those in the most posterior follicles).

In this study, ovaries were classified into one of seven stages as follows: early previtellogenesis, late previtellogenesis, early vitellogenesis, mid vitellogenesis, late vitellogenesis, ovaries just mature, fully mature ovaries. This scoring system distinguished three stages of vitellogenesis (early, mid, and late) rather than the two (early and late) scored in Kambysellis and Craddock (1991). The mid vitellogenic stage corresponded to stage S10 as described by King (1970) for *D. melanogaster.*

Rate of Ovarian and Sexual Maturation in D. silvestris *Females under Laboratory Conditions*

Newly eclosing F_1 adults of the Y-11 strain maintained at UH were collected daily at 1600 hours over an 8-day period. A total of 159 males and 141 females was obtained. Flies from each day's collection were immediately set up with food vials in a 3.785-liter jar and maintained at 17–18°C on a 10:14 hr (L:D) cycle. After several days aging, the live males were removed for another experiment; all dead and deformed flies were also removed. The remaining live Y-11 females of defined age were counted, and the sex ratio in each jar was restored to 1:1 by addition of the requisite number of males of the laboratory strain YR$, derived from the same natural population in the Upper ʻŌlaʻa Forest Reserve. Because eclosing adults of this latter strain were collected only biweekly, precise ages of these males were not known, in contrast with the case for the females.

The eight jars were maintained in the Drosophila rearing room at UH with changes of food vials every 5 days. The insemination status and ovarian developmental stage of females from 14 to 21 days of age were monitored by withdrawing an average of two flies per jar per day for dissection and microscopic analysis of their ovaries and sperm storage organs. This sampling procedure, spreading each female age sample across all jars, was designed to avoid any unusual effects on female maturation within an individual jar due to the particular group of males present within that jar.

RESULTS

Table 1 presents the ovarian developmental profile and insemination status of 83 wild-caught unmarked females sampled from the *D. silvestris* population in the Upper ʻŌlaʻa Forest Reserve. About 40% of the females from this population were sexually mature, as judged by the fact that they had been inseminated. The earliest ovarian stage at which insemination was observed in these field flies was mid vitellogenesis. In the two females inseminated at that stage, mating must have occurred only recently, within the few hours immediately before their capture. In both females, the uterus was distended with a large mass of sperm that was still in the process of being packaged into the seminal receptacle and spermathecae for storage. One of the three females at late vitellogenesis had likewise been recently inseminated. Another of the females with late vitellogenic ovaries was captured at an established lek

TABLE 1

OVARIAN STAGE AND INSEMINATION STATUS[a] OF WILD-CAUGHT FEMALES

	OVARIAN STAGE							
COLLECTION DATE (1987)	EARLY PREVIT.	LATE PREVIT.	EARLY VIT.	MID VIT.	LATE VIT.	JUST MATURE	FULLY MATURE	*n*
28 May	3 (0)	2 (0)	2 (0)	2 (0)	2 (2)	1 (1)	3 (3)	15
6 June	1 (0)	5 (0)	1 (0)	1 (1)	—	—	2 (2)	10
7 June	1 (0)	1 (0)	3 (0)	—	—	1 (1)	2 (2)	8
8 June	—	—	6 (0)	1 (0)	—	3 (2)	3 (3)	13
11 June	—	1 (0)	1 (0)	—	—	—	—	2
12 June	3 (0)	2 (0)	1 (0)	1 (0)	—	1 (1)	4 (4)	12
13 June		2 (0)	2 (0)	1 (0)	—	—	2 (2)	7
? June	2 (0)	—	1 (0)	4 (1)	—	1 (1)	5 (5)	13
? June	—	—	—	1 (0)	1 (1)	—	1 (1)	3
Total	10 (0)	13 (0)	17 (0)	11 (2)	3 (3)	7 (6)	22 (22)	83

[a] Numbers within parentheses are the number of females at that ovarian developmental stage that were inseminated.

site; it probably had mated 7 or more hours previously, as judged by the fact that the uterus was no longer distended and all transferred sperm had been stored (E.M.C., unpubl. obs.). Of the seven females with ovaries that were just maturing, only one had not been inseminated. All of the older females with fully mature ovaries had mated and stored an abundant supply of sperm for fertilization of their numerous eggs.

Of the young marked adults of *D. silvestris* released at the study site, 84 females were recaptured. Estimated ages of these females upon recapture ranged from approximately 7 days to over 4 months, and all ovarian developmental stages were included in the sample (Table 2). Because eclosing adults to be marked were not collected daily, exact eclosion dates were not known and therefore the ages of individual females upon recapture could not be more precisely determined.

Figure 1*C* shows the profile of ovarian development as a function of chronological age in the sample of marked females. Because the numbers are small, the ovarian stages have been grouped into three categories: previtellogenesis, vitellogenesis, and mature ovaries. In the field, females with previtellogenic ovaries were recovered up to an age of 24 ± 2 days. Vitellogenic females ranged in age from 14 ± 2 to 34 ± 4 days. From 35 days of age onward, all recaptured females had just mature or fully mature ovaries; furthermore, all these older females had been inseminated (Table 2).

Of the 60 females recaptured at ages less than 35 days, only 10 were inseminated, with the chronologically earliest insemination being recorded at 18 ± 6 days. Developmentally, the earliest mating occurred at mid vitellogenesis in a female 34 ± 4 days old, and again insemination had recently occurred in the hours just before capture. The five younger females with mid vitellogenic ovaries and all developmentally less mature females were uninseminated. As in the sample of unmarked females, the few at the brief stage of late vitellogenesis had been inseminated (one just recently), as had most of the females that had just matured their first few eggs. The two exceptions among the females with just maturing ovaries were 26 ± 2 and 31 ± 6 days old, at the younger end of this developmental stage.

Table 3 presents the ovarian stage and insemination data for laboratory-reared flies derived from the same *D. silvestris* population. Although reared to adult eclosion equivalently to those that were marked and released into the field, these F_1 females were collected daily following eclosion, so that there is no more than 24 hr variability in sample age. Also, these adults were closely confined in jars on laboratory medium under conditions of constant and higher ambient temperatures than those encountered by flies in the field. Dissections were performed only within the range of 14–21 days adult age because prior laboratory studies of this species had indicated this age range as the likely period of female reproductive maturation under laboratory conditions. Thus the age range of this sample is much narrower than that of the marked, released, and recaptured sample.

In these laboratory-reared *D. silvestris* females, vitellogenesis began at ages anywhere from less than 14 days up to 20 days (Table 3, Figure 1*A*), demonstrating substantial variability in developmental rates among individuals, even under constant conditions. Ovarian maturation was achieved over a similar time range from 15 days onward. Earliest inseminations occurred at mid vitellogenesis, with 11.5% of females at that developmental age inseminated. Insemination rates increased with increasing developmental stage, but did not reach 100%, even in the females with fully mature ovaries.

Because of the differences in ambient temperature experienced by flies in Upper ʻŌlaʻa Forest Reserve versus the UH laboratory, and the obvious effect of temperature on developmental rates, another group of flies was maintained in captivity at Volcano near the study site. Here temperatures were more nearly comparable with those at the field study site, but the flies' nutrition was that provided in the laboratory, not that available in the field. As with the flies released into the field, ages of individuals were tracked by marking the flies before adding them to the

TABLE 2

AGE, OVARIAN STAGE, AND INSEMINATION STATUS[a] OF RECAPTURED FEMALES

	OVARIAN STAGE							
AGE (days)	EARLY PREVIT.	LATE PREVIT.	EARLY VIT.	MID VIT.	LATE VIT.	JUST MATURE	FULLY MATURE	*n*
7 ± 2	6 (0)	—	—	—	—	—	—	6
8 ± 3	3 (0)	—	—	—	—	—	—	3
10 ± 3	2 (0)	2 (0)	—	—	—	—	—	4
11 ± 2	1 (0)	1 (0)	—	—	—	—	—	2
12 ± 3	5 (0)	1 (0)	—	—	—	—	—	6
12 ± 5	—	1 (0)	—	—	—	—	—	1
14 ± 2	—	2 (0)	1 (0)	—	—	—	—	3
14 ± 6	2 (0)	1 (0)	—	—	—	—	—	3
15 ± 2	—	2 (0)	3 (0)	—	—	—	—	5
15 ± 6	2 (0)	—	—	—	—	—	—	2
17 ± 6	—	1 (0)	—	—	—	—	—	1
18 ± 3	—	—	1 (0)	—	—	—	—	1
18 ± 6	—	—	—	—	—	1 (1)	—	1
19 ± 6	—	2 (0)	—	—	—	—	—	2
20 ± 6	—	1 (0)	—	—	—	—	—	1
22 ± 2	—	—	—	—	1 (1)	—	—	1
24 ± 2	—	1 (0)	—	1 (0)	—	2 (2)	—	4
24 ± 6	—	—	—	1 (0)	—	—	—	1
25 ± 2	—	—	—	—	—	1 (1)	—	1
26 ± 3	—	—	—	1 (0)	—	1 (0)	—	2
27 ± 6	—	—	—	1 (0)	—	—	—	1
31 ± 6	—	—	—	—	2 (2)	1 (0)	—	3
32 ± 5	—	—	—	1 (0)	—	1 (1)	—	2
34 ± 6	2 (0)[b]	—	—	1 (1)	—	1 (1)	—	4
35 ± 4	—	—	—	—	—	1 (1)	1 (1)	2
38 ± 5	—	—	—	—	—	—	1 (1)	1
40 ± 4	—	—	—	—	—	2 (2)	1 (1)	3
42 ± 3	—	—	—	—	—	—	2 (2)	2
46 ± 4	—	—	—	—	—	1 (1)	1 (1)	2
47 ± 3	—	—	—	—	—	—	1 (1)	1
48 ± 4	—	—	—	—	—	2 (2)	2 (2)	4
53 ± 3	—	—	—	—	—	1 (1)	1 (1)	2
55 ± 3	—	—	—	—	—	1 (1)	—	1
63 ± 4	—	—	—	—	—	—	1 (1)	1
72 ± 4	—	—	—	—	—	—	1 (1)	1
76 ± 4	—	—	—	—	—	—	1 (1)	1
88 ± 3	—	—	—	—	—	—	1 (1)	1
>108	—	—	—	—	—	—	1 (1)	1
>121	—	—	—	—	—	—	1 (1)	1
Totals	23 (0)	15 (0)	5 (0)	6 (1)	3 (3)	16 (14)	16 (16)	84

[a] Numbers within parentheses are the number of females at that ovarian developmental stage that were inseminated.
[b] These two females had abnormal, rudimentary ovaries and were probably female-sterile.

container. In this small sample of "field-reared" flies, all ovarian stages except early previtellogenesis were observed in females ranging in age from 16 ± 2 to 66 ± 1 days of age (Table 4). Vitellogenic females were from 16 ± 2 to 35 ± 2 days of age; females with just maturing ovaries were first observed at 27 days (Figure 1*B*). The youngest developmental age at which inseminations took place was again mid vitellogenesis, and the youngest recorded chronological age at first insemination was 21 ± 1 days (Table 4).

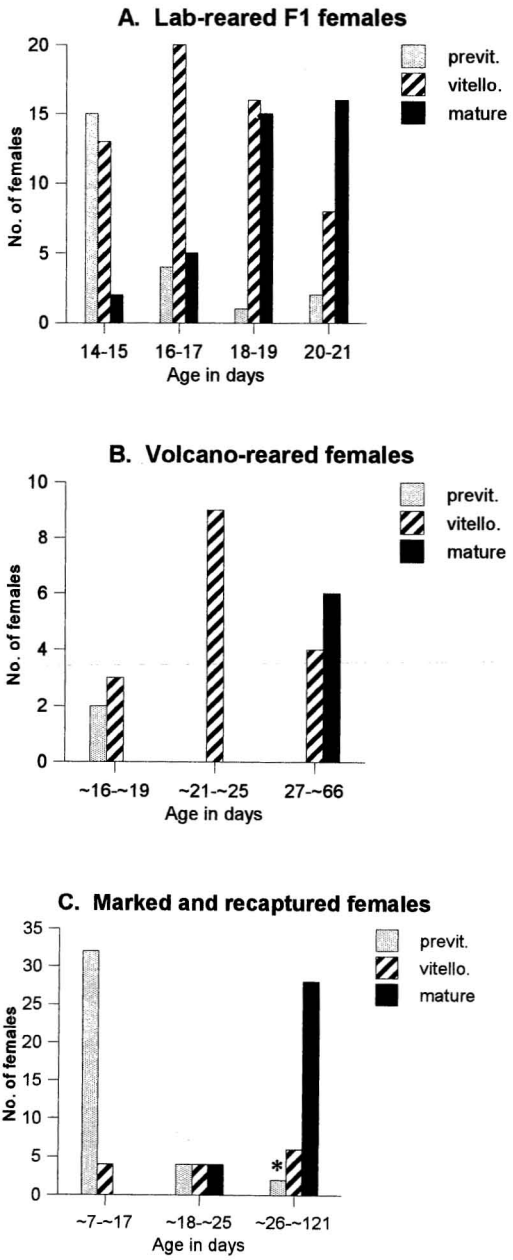

FIGURE 1. Age profile of ovarian development in *D. silvestris* females from the Upper 'Ōla'a Forest Reserve population. Ovarian stages are grouped into three categories: previtellogenic (lightly shaded bars), vitellogenic (hatched bars), and mature (solid bars). *A.* Sample of 117 laboratory-reared F_1 females (14–21 days). *B.* Sample of 24 females reared in captivity outdoors at Volcano (~16–66 days). *C.* Sample of marked, released, and recaptured females (from ~7 days up to ~121 days). The bar with an asterisk corresponds to two females with abnormal, rudimentary ovaries that were judged to be female-sterile.

Table 5 summarizes insemination frequencies in all four samples of females analyzed. Here, the late and mid vitellogenic stages were combined because so few females were sampled at late vitellogenesis. Figure 2 presents a graphical comparison of the ovarian stage distributions in the four samples analyzed; age ranges of each sample are indicated in the caption. The late vitellogenic stage is the least frequent, consistently composing 4% of each sample. Temporally, it is the briefest stage in ovarian maturation.

The two samples collected at baits from the field population (Figure 2*A,B*) include all seven ovarian stages, with a sizable proportion of young females in previtellogenic stages. All females in the other two "reared" samples (Figure 2*C,D*) are at least 2 weeks old and well beyond early previtellogenesis, which is therefore not represented in those samples. Furthermore, they comprise a narrower age range than the samples shown in Figure 2*A* and *B* and so include proportionately fewer fully mature females and more at vitellogenic stages.

DISCUSSION

Pattern of Female Reproductive Maturation

Upon eclosion, the ovaries of *D. silvestris* females are small and undeveloped (previtellogenic), remaining so for the first 2 or more weeks of adult life. Overall, the ontogeny of ovarian maturation under field conditions (Figure 1*B,C*) is similar to that in the laboratory (Figure 1*A*), except that the rate and duration differ markedly and field females show much more variability in developmental rates. These differences can be attributed in part to lower ambient temperatures in nature and the fact that nutrient availability is less predictable. Furthermore, the maturing of eggs is an energy-expensive process. Whereas laboratory females confined in a jar have food continuously available, in the field developing females must spend more of their energy budget on flying in search of feeding sites. This activity inevitably reduces the energy available for physi-

TABLE 3

AGE, OVARIAN STAGE, AND INSEMINATION STATUS[a] OF LABORATORY-REARED FEMALES

	OVARIAN STAGE							
AGE (days)	EARLY PREVIT.	LATE PREVIT.	EARLY VIT.	MID VIT.	LATE VIT.	JUST MATURE	FULLY MATURE	*n*
14	—	8 (0)	3 (0)	3 (1)	—	—	—	14
15	—	7 (0)	4 (0)	2 (0)	1 (0)	2 (0)	—	16
16	—	2 (0)	5 (0)	6 (1)	—	1 (0)	—	14
17	—	2 (0)	4 (0)	4 (0)	1 (1)	4 (3)	—	15
18	—	1 (0)	2 (0)	5 (1)	2 (0)	6 (3)	—	16
19	—	—	4 (0)	3 (0)	—	6 (5)	3 (2)	16
20	—	2 (0)	4 (0)	3 (0)	1 (1)	6 (5)	1 (1)	17
21	—	—	—	—	—	6 (3)	3 (2)	9
Totals	—	22 (0)	26 (0)	26 (3)	5 (2)	31 (19)	7 (5)	117

[a]Numbers within parentheses are the number of females at that ovarian development stage that were inseminated.

TABLE 4

AGE, OVARIAN STAGE, AND INSEMINATION STATUS[a] OF FEMALES REARED IN CAPTIVITY AT VOLCANO

	OVARIAN STAGE							
AGE (days)	EARLY PREVIT.	LATE PREVIT.	EARLY VIT.	MID VIT.	LATE VIT.	JUST MATURE	FULLY MATURE	*n*
16 ± 2	—	1 (0)	1 (0)	1 (0)	—	—	—	3
19 ± 1	—	1 (0)	1 (0)	—	—	—	—	2
21 ± 1	—	—	1 (0)	—	1 (1)	—	—	2
23 ± 1	—	—	1 (0)	2 (2)	—	—	—	3
25 ± 2	—	—	3 (0)	1 (1)	—	—	—	4
27 ± 0	—	—	1 (0)	2 (2)	—	1 (1)	—	4
28 ± 1	—	—	—	—	—	1 (1)	—	1
35 ± 2	—	—	—	1 (1)	—	—	1 (1)	2
66 ± 1	—	—	—	—	—	2 (2)	1 (1)	3
Totals	—	2 (0)	8 (0)	7 (6)	1 (1)	4 (4)	2 (2)	24

[a]Numbers within parentheses are the number of females at that ovarian developmental stage that were inseminated.

ological maturation of the ovaries. With the onset of vitellogenesis, there is massive synthesis of vitellogenins or yolk proteins. The amounts made in a 24-hr period in a related Hawaiian *Drosophila, D. grimshawi,* represented over 2% of the fly's body weight under laboratory conditions and constituted some 40% of the total blood proteins before their uptake into the ovaries (Kambysellis et al. 1989).

Comparing field and laboratory females of *D. silvestris,* the range of ages at initiation of vitellogenesis is not markedly different: <14–21 days for laboratory females (Table 3) versus 14 ± 2 to 24 ± 6 days in the marked, recaptured flies (Table 2) and 16 ± 2 to 21 ± 1 days in the small sample of Volcano-reared flies (Table 4), showing interindividual variability in all cases. It should be noted, however, that all the marked flies had eclosed in the laboratory and been maintained on laboratory medium for approximately the first week of their adult life.

Although times of onset of vitellogenesis are reasonably comparable, age at maturation of the first eggs was more variable in

TABLE 5

PERCENTAGE OF INSEMINATION IN *D. silvestris* FEMALES AS A FUNCTION OF OVARIAN DEVELOPMENTAL STAGE

	OVARIAN STAGE							
SAMPLE	EARLY PREVIT.	LATE PREVIT.	EARLY VIT.	MID–LATE VIT.	JUST MATURE	FULLY MATURE	*n*	YOUNGEST AGE INSEMINATED (days)
Wild-caught	0	0	0	35.7	85.7	100.0	83	—
Marked, released, and recaptured	0	0	0	44.4	87.5	100.0	84	18 ± 6
Laboratory-reared	—	0	0	16.1	61.3	71.4	117	14
Volcano-reared	—	0	0	87.5	100.0	100.0	24	21 ± 1
No. of females	33	52	56	62	58	47	308	

the forest (18 ± 6 to 55 ± 3 days [Table 2]) than in the laboratory (15–21 days [Table 3]). Thus rates of vitellogenesis and egg maturation were substantially slower and more variable in the natural population. After the first eggs mature, vitellogenesis continues as oviposition ensues and oocytes in younger follicles move down the ovarioles. If a suitable oviposition substrate is not found immediately, hundreds of mature eggs can be stored in the ovaries even as additional oocytes continue their maturation.

Females can be judged to be sexually mature once they respond to the courtship overtures of conspecific males. In *D. silvestris,* the insemination data (Table 5) are consistent in showing that no females were receptive to insemination before mid vitellogenesis, either in the laboratory or in the field. From mid vitellogenesis onward, insemination frequencies increased as the ovaries matured. It is notable that the handling and marking of the released flies did not interfere with their mating success; they showed the same ovarian maturation pattern and insemination profile as the wild-caught flies. The current data confirm and extend earlier observations on the sexual maturation of *D. silvestris* females in the field (Kambysellis and Craddock 1991) and in the laboratory (Craddock and Boake 1992), and further support the correlation already noted between the onset of female receptivity and the process of vitellogenesis (Kambysellis and Craddock 1991). Apparently, both share a common physiological basis, and the reproductive physiology of these flies in the laboratory is no different from that in nature, except in rate.

It is interesting that the mating success of field females, both marked and unmarked, exceeded that of the laboratory females at all competent ovarian stages (Table 5). In fact, all field-collected females with fully mature ovaries were inseminated. This implies that all female individuals that reach reproductive maturity in a *D. silvestris* population ultimately mate and thus potentially contribute offspring to the next generation. There may be minor differences in reproductive fitness among females surviving to adulthood, but none were detectable in this analysis; possibly selection acts more intensely on earlier life history stages.

Despite the continuous availability of sexually mature males, the laboratory-reared females showed markedly lower frequencies of insemination (Tables 3, 5), conceivably because of the complexity of mating behavior in this species (specifically the lek behavior of males [Spieth 1978]). Although females were physiologically mature and presumably sexually receptive, courtship and mating were apparently inhibited within the confines of the 3.785-liter jar. By contrast, the females held with males at Volcano in more spacious accommodations (in a garbage can) were inseminated comparably with the field females (Tables 4, 5).

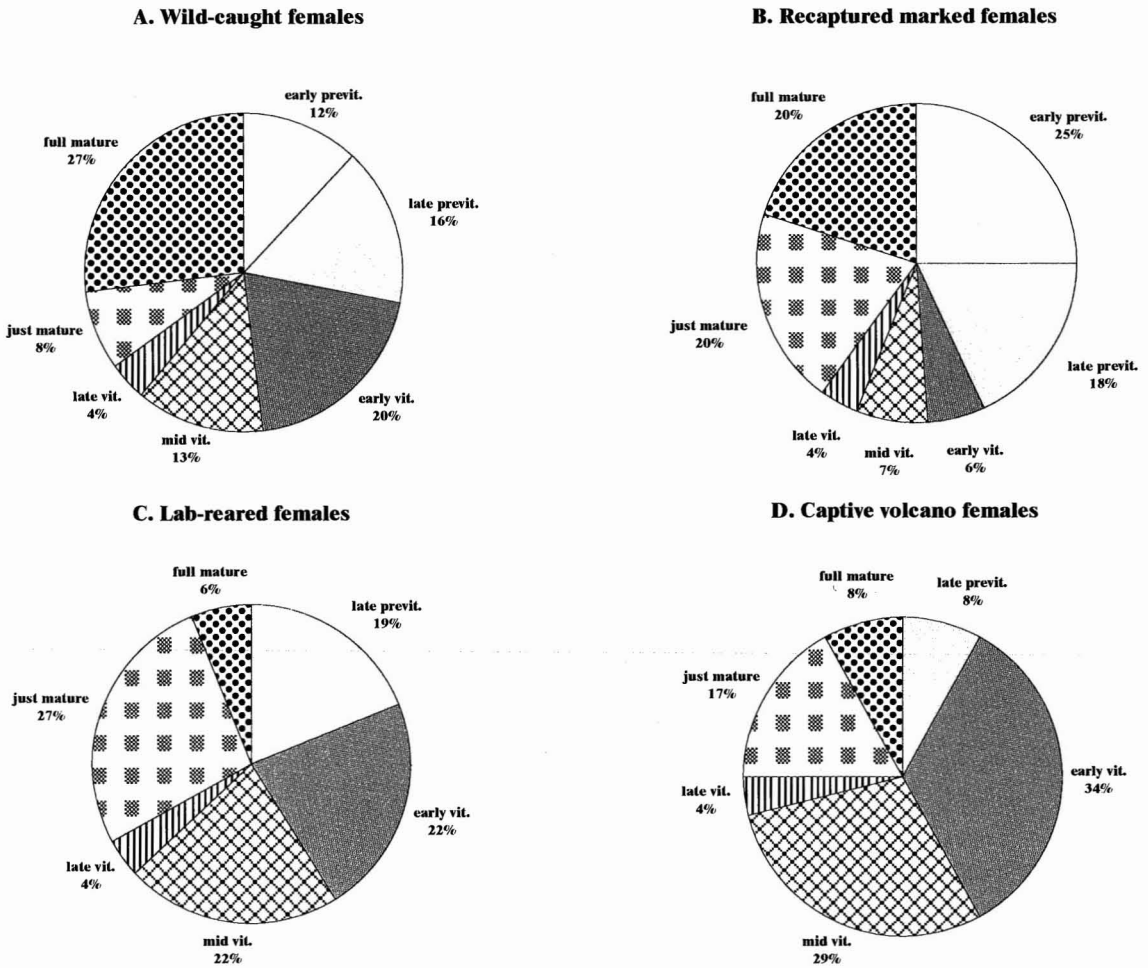

FIGURE 2. Frequency distributions of ovarian developmental stages in the four samples of *D. silvestris* females, categorized by the seven developmental stages recognized in this study. Samples *C* and *D* included representatives of only six ovarian stages because they lacked very young flies in early previtellogenesis. *A.* Pattern of reproductive maturation in sample of 83 wild-caught females of unknown age. *B.* Sample of 82 recaptured, marked flies, 7 ± 2 to >121 days of age. *C.* Sample of 117 laboratory-reared flies, 14–21 days old. *D.* Females (24) held in captivity at Volcano, 16 ± 2 to 66 ± 1 days old.

Alternatively, it could be argued that not all matings that took place in the laboratory jars were detected, because some of the males may have failed to transfer sperm. Schwartz (1991) showed that over half of *D. silvestris* males that mated twice within 48 hr were depleted of sperm and temporarily sterile, even though they were still able to copulate. This explanation for the apparently lower insemination frequencies of laboratory-reared females cannot be ruled out. In the field, where population densities and mating frequencies are lower, such temporary reductions in male fertility are less likely.

Population Age and Breeding Structure

Data on the reproductive status of field-collected females together with knowledge of the rate and pattern of ovarian maturation in

D. silvestris can be applied to assess the adult age distribution in a natural population. A similar evaluation of males is much less useful because they mature more rapidly (Boake and Adkins 1994) and provide fewer developmental markers. The presence of sperm in the testes is not particularly informative because this was observed in dissections of marked, recaptured males ranging in age from 8 ± 5 to 127 ± 4 days of age. Even small size of the paragonial glands is not indicative of age because this occurs in old males that have just mated, as well as in young and reproductively immature males. In maturing females of this species, by contrast, the ovaries progress through distinctive developmental stages, albeit at variable rates in field individuals.

Considering the developmental rates of the marked, recaptured females as representative of those in the Upper ʻŌlaʻa population, we can estimate that the wild-caught flies showed an approximate age distribution as follows: 28% were young flies no more than 2 to 3 weeks from eclosion and in various stages of previtellogenesis; some 37% were maturing flies from 2 to 4 or more weeks of age with their ovaries in active vitellogenesis; and 35% were mature adults from 1 to more than 4 months old. The majority of the mature females were older flies, composing about one-fourth of the total adult population.

Because of the adult longevity of this species, and the maturation rate, one might have expected the adult population typically to include about three-fourths older, fully mature individuals. Thus the excess of young flies (65%) up to 4 or 5 weeks old (Table 2, Figures 1 and 2) needs some explanation. Because the samples were collected by baiting, the age profile could have been distorted by the greater attractiveness of the baits to the younger, maturing flies that, conceivably, have higher nutritional needs. But old females that continue to mature large numbers of eggs must have equivalent nutritional needs. If real, this age distribution may suggest active predation of the adult population by birds and/or wasps, with an individual's probability of becoming prey increasing as a function of age. Alternatively, the excess of younger flies may indicate that this particular population was in a flush phase (Carson 1968) as a result of the fact that a suitably rotting *Clermontia* or alternative host plant had become available as an oviposition substrate some 3 to 6 months previous to collection of these adult samples.

The preponderance of young flies up to 4 or 5 weeks old among the recaptured *D. silvestris* could also be explained as a dispersal effect, with distance dispersed being a function of time. Moreover, dispersal may depend on life history stage, and females may not begin active displacement until they need to search for an oviposition substrate, after they have mated and their ovaries have matured (i.e., after they are 4 or 5 weeks old). It is important to note, however, that in this study, the majority of flies were recaptured at baits, and baits provide the flies with a strong cue to remain in an area. As already noted by Richardson and Johnston (1975) in their studies of dispersal in another Hawaiian fly, *D. mimica,* baits can seriously bias dispersal patterns. The persistence of some marked individuals in the study area for as long as 4 months tends to support the likelihood of a baiting effect. On the other hand, because there was a sizable resident population in this area, it must have had suitable breeding substrates available, which may have explained the relative lack of movement of some of the older flies. These various alternatives can only be evaluated by further sampling and analysis of this and other *D. silvestris* populations.

Whereas all reproductively mature females in the ʻŌlaʻa Forest population mated, the same probably does not apply to males. Because of their lek behavior (Spieth 1978), males of this species are subject to intense intrasexual selection (Carson 1997). Their variable reproductive success in the laboratory is evident from the fact that, in a cage situation, one-third of actively courting males fail to copulate, and another third accomplish about two-thirds of the observed matings (Spiess and Carson 1981, Carson 1987). This differential mating pattern in laboratory populations has been found to

correlate with genotype in that heterokaryotypic individuals showed higher mating success in both males and females, but especially in males (Carson 1987). In nature, competition and sexual selection among males are likely to be more intense than in the laboratory, with only a proportion of the most highly fit adult males participating in reproduction, thus reducing the effective breeding size of the population. The current data provide no evidence, however, that the breeding females are similarly restricted to a subset of the surviving adults. Thus the breeding structure of the population is not the same for the two sexes; whereas 100% of the females mate, only a fraction of the males do so, with some individuals being more successful than others.

Although it is not possible to estimate from a female's sperm reserves how often she has mated, genetic data indicate that, by contrast with males, only a few *D. silvestris* females in a population engage in multiple mating (Craddock and Johnson 1978). No old females lacking sperm were found in either the marked or the unmarked samples from the ʻŌlaʻa population. This suggests that sufficient sperm may be transferred in the initial mating to fertilize all the eggs produced in a female's lifetime. In nature females of this species apparently have a propensity to remate, but may only do so when their sperm supply is close to depletion.

The data presented here show that analyzing the reproductive status of females in a *Drosophila* population such as that of *D. silvestris* can augment our understanding of population parameters such as age distribution and breeding structure. Developmental data are particularly useful when the species is long-lived as in this case. If something is known about the stage of sexual receptivity in the course of maturation, then insemination data can also illuminate the female contribution to the effective breeding population.

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